REMARKS

Claims 1–4, 6, and 8–24 are pending in the application and have been examined. Claims 1-4, 6, and 8-24 stand rejected. Claims 1 and 21 have been amended. Claim 18 has been canceled. Reconsideration and allowance of Claims 1–4, 6, 8-17 and 19-24 is respectfully requested.

The Rejection of Claims 1, 3–4, 6, 8, 10, 12, 15, and 18–21 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Brownie et al., *Nucleic Acids Research*, 25(16):3235–3251 (1997) in View of Lau et al., *Science 294*:858–862 (2001), as Evidenced by Lau, Supplemental Information

Claims 1, 3–4, 6, 8, 10, 12, 15, 18–21 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Brownie et al., *Nucleic Acids Research 25*(16):3235-3251 (1997) ("Brownie") in view of Lau et al., *Science 294*:858–862 (2001) (and Supplemental Information) ("Lau"). Applicant respectfully traverses this ground of rejection for at least the following reasons.

While not acquiescing to the Examiner's position, but in order to clarify the invention, Claim 1 and independent Claim 21 have been amended to incorporate the limitation of original Claim 18, now canceled. Claims 1 and 21 as amended now recite at step (b) and step (2), respectively, "amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions." Support for this amendment is found in original Claim 18, now canceled, and in the specification at page 8, line 7 to page 9, line 14, Table 1, Table 2, Table 6 and Figure 1.

The cited references, either alone or in combination, fail to render the claimed invention unpatentable. KSR confirmed that the Grahm Factor Analysis should be used in determining whether a claimed invention is obvious under Section 103(a). *KSR Int'l Co. v. Teleflex Inc.*, 127

S.Ct. 1727, 1739 (2007). This analysis includes assessing the rejected claims, the scope and content of the cited art, and the differences between the rejected claims and the cited art. Id. at 1734. As will be shown, a *prima facie* case of obviousness has not been established because (1) the references taken together or separately, fail to teach every limitation of the claimed invention; (2) there is no motivation or expectation of success to combine the references to arrive at the claimed invention because the hypothetical combination proposed by the Examiner would render the method of Brownie inoperable for its intended purpose; and (3) the hypothetical combination proposed by the Examiner does not result in the claimed invention.

The inquiry under *Graham* includes ascertaining the differences between the prior art and the claims at issue.

1. The Differences between the Rejected Claims, as amended and the Cited Art

Brownie et al.

The Examiner admits that Brownie does not teach a primer extension reaction carried out on a microRNA target. It is further noted that Brownie does not teach or suggest producing a first DNA molecule that is complementary to a target microRNA molecule. Further, Brownie does not teach or suggest amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward primer and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions.

In sharp contrast to the claimed invention, which is directed to methods for quantitating specific target microRNAs, Brownie is generally directed to the detection of primer dimers and methods and reagents used to eliminate primer-dimer accumulation in PCR assays, and is silent with respect to microRNAs.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Lau et al.

The Examiner admits that Lau et al. does not teach the use of an extension primer comprising a first portion that hybridizes to a target microRNA. See Office Action mailed June 24, 2009, at page 11. It is further noted that Lau et al. does not teach or suggest amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward primer and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions.

Rather, in sharp contrast to the claimed invention, which is directed to methods for *quantitating specific target microRNAs*, Lau is directed to the *non-specific* amplification of *all microRNAs in a sample* through the ligation of adaptor linkers for the purpose of cloning *previously unidentified* microRNA species. In particular, Lau et al. describes the cloning of endogenous C. elegans miRNAs and the discovery of 55 previously unknown miRNAs in C. elegans. Abstract. Lau describes the construction of an amplified small RNA library by first ligating 3' RNA adaptor oligonucleotides to a pool of gel-purified 18-26nt small RNAs from mixed-stage worms with T4 RNA ligase, gel purifying the ligated RNA, then ligating to a 5' adaptor oligonucleotide in a second T4 RNA ligase reaction, gel purifying the products from the second ligation followed by reverse transcription and PCR amplification of the linker-ligated products using DNA oligos *corresponding to the adaptor sequences*. The PCR products were submitted for sequencing. See Footnote 23 and Lau supplementary materials.

Moreover, Lau et al. does not remotely teach or suggest that the above described cloning method could be used for measuring the amount of a target microRNA in a sample. Rather, in contrast to the claimed methods, Lau et al. teaches the use of Northern blots for measuring microRNA expression. See pages 859 and 861, and FIGURE 3.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS[PLLC]
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

2. The Differences between the Rejected Claims and the Cited Art Are Not Obvious Differences

There is no apparent reason to combine Brownie and Lau as asserted by the Examiner. In the context of an obviousness rejection, the Supreme Court explained the importance of "identifying a reason" why a skilled artisan would be prompted to arrive at the presently claimed invention. *KSR*, 127 S.Ct. at 1741. The Court noted that there should be an "explicit" analysis regarding "whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." Id. Because the combination of references fails to teach every limitation of the present claims, there can be no "apparent reason" to combine the references to arrive at the claimed invention. It is noted that the combination of references would not result in the claimed invention because there is no ability in the methods disclosed in the cited references to quantitatively measure a particular target microRNA as claimed. Rather, Lau teaches the *non-specific* amplification of *all microRNAs in a sample*, and Brownie et al. teaches methods to *eliminate primer-dimer accumulation* in a PCR reaction.

Moreover, even if the teachings of Brownie and Lau were to be combined, such a combination would not result in the claimed invention, where an extension primer that specifically hybridizes to a target microRNA is used to amplify microRNA. As noted above, neither Brownie nor Lau teach or suggest the use of an extension primer comprising a first portion that hybridizes to a target microRNA. Furthermore, neither Brownie nor Lau teach or suggest amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward primer and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

It is further noted that the Examiner's proposed treatment of the prior art to have applied the format of tagged and tailed primers of Brownie to the non-specific detection of microRNA sequences as taught by Lau would actually render the method of Brownie unsatisfactory for its intended purpose of detecting and eliminating primer dimers. Brownie specifically teaches that the use of the same Tail sequence for each Tail primer enables the use of a single Tag primer as both the forward and reverse primer for amplification of the product. The advantage of the Brownie method is that after genomic priming with Tail primers, any primer dimers that form will produce molecules with complementary ends, resulting in the preferential hairpinning of the short molecules ("pan-handle" formation) over amplification. See Brownie et al., Figure 2B and page 3235. Any modification of Brownie to incorporate microRNA specific primers, as proposed by the Examiner, would prevent pan-handle formation, thus destroying the primary intended purpose of the Brownie method (i.e., to eliminate primer-dimer accumulation in PCR reactions). Therefore, it is demonstrated that there is no motivation to modify Brownie as proposed by the Examiner. See M.P.E.P § 2143.01 ("If [a] proposed modification would render the prior art invention being modified unsatisfactory to its intended purpose, then there is no suggestion or motivation to make the proposed modification."). Further, as noted above, Lau does not teach the use of microRNA specific primers, but rather *non-specific* amplification of *all microRNAs in a sample.*

In view of the foregoing, it is demonstrated that a *prima facie* case of obviousness has not been established because Brownie and Lau, taken together or separately, fail to teach every limitation of Claims 1 and 21. Furthermore, it is demonstrated that a person of ordinary skill in the art would not modify Brownie to arrive at the present invention as recited in Claims 1, (from which Claims 3–4, 6, 8, 10, 12, 15, and 19–20 depend), and Claim 21. Accordingly, removal of this ground of rejection is respectfully requested.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

The Rejection of Claims 2, 13–14, 16–17, and 22 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Brownie et al., *Nucleic Acids Research 25*(16):3235–3251 (1997) in View of Lau et al., *Science 294*:858–862 (2001), as Evidenced by Lau Supplemental Information, as Applied Above, in Further View of Braasch et al., *Chemistry & Biology 8*:1–7 (2001)

Claims 2, 13–14, and 16–17, which depend from Claim 1, and Claim 22, which depends from Claim 21, stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Brownie in view of Lau, in further view of Braasch et al., *Chemistry & Biology 8*:1–7 (2001) ("Braasch"). Applicant respectfully traverses this ground of rejection for at least the following reasons.

Claims 1 and 21 have been amended as described supra. As described above with respect to Claims 1 and 21, Brownie and Lau, either separately or together, fail to teach or suggest every element of the claimed invention. Further, no motivation exists to combine these references and there is no reasonable expectation of success to achieve the claimed invention with respect to such a hypothetical combination. The teachings of Braasch fail to remedy the deficiencies of Brownie and Lau.

The Examiner cites Braasch as teaching that locked nucleic acids are nucleic acid analogs that may be incorporated into DNA and RNA oligomers. It is noted that there is no remote teaching or suggestion in Braasch with regard to the use of an extension primer comprising a first portion that hybridizes to a target microRNA, as claimed. It is further noted that Braash does not teach or suggest amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward primer and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions, as claimed.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS[PLLC]
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Thus, as with independent Claims 1 and 21, these dependent claims are not obvious over the cited art. Accordingly, removal of this ground of rejection is respectfully requested.

The Rejection of Claims 23–24 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Brownie et al., *Nucleic Acids Research 25*(16):3235–3251 (1997) in View of Lau et al., *Science 294*:858–862 (2001), as Evidenced by Lau Supplemental Information, as Applied Above, in Further View of U.S. Patent Publication No. 2003/0186288 (Spivack et al.)

Claims 23–24, which depend from Claim 21, stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Brownie in view of Lau, in further view of U.S. Patent Publication No. 2003/0186288 ("Spivack"). Applicant respectfully traverses this ground of rejection for at least the following reasons.

Claim 21 has been amended as described supra. As described above with respect to independent Claim 21, from which Claims 23–24 depend, Brownie and Lau, either separately or together, fail to teach or suggest every element of the claimed invention. Further, no motivation exists to combine these references and there is no reasonable expectation of success to achieve the claimed invention with respect to such a hypothetical combination. The teachings of Spivack fail to remedy the deficiencies of Brownie and Lau.

The Examiner characterizes Spivack as teaching the amount of amplified DNA molecules being measured by fluorescence-based quantitative PCR, including an embodiment wherein the amount of amplified DNA molecules are measured using SYBR green dye. It is noted that there is no remote teaching or suggestion in Spivack with regard to the use of an extension primer comprising a first portion that hybridizes to a target microRNA, as claimed. Rather, Spivack et al. discloses the use of an RT-PCR strategy that detects mRNA with a primer that hybridizes to the polyA tail of the mRNA. See, for example, paragraph [0103] of Spivack, which states,

"The methods of the present invention can be used to analyze gene expression form any material in which genes are expressed to generate mRNA molecules having poly-A tails." As described in paragraph [0057] of Spivack, the RT primers used to perform reverse transcription comprise (a) a 3' anchor sequence; (b) a poly T midsection to anneal to the polyA tail of the mRNA; and (c) a 5' tag. Spivack et al. does not disclose or suggest a method using an extension primer that specifically hybridizes to a target microRNA as claimed, because, as known by those of skill in the art, a mature microRNA does not contain a polyA tail.

Further, the combination would not result in the claimed invention because there is no ability in any of the cited references to quantitatively measure a particular target microRNA. In sharp contrast to the claimed invention, which is directed to methods for quantitating specific target microRNAs, the teachings of Lau and Spivack are both directed to the non-specific amplification of either all microRNAs in a sample through the ligation of adaptor linkers (Lau), or through the use of oligo-dT priming of all polyA+ mRNA (Spivack). Thus, no combination of the cited references renders the claimed invention obvious. See MPEP § 2143.02 and *KSR*, 127 S.Ct. at 1741.

As noted above, contrary to the Examiner's contention, Spivack describes the use of an RT-PCR strategy that detects mRNA with a primer that hybridizes to the polyA tail of the mRNA. Spivack does not teach or suggest a method comprising a primer that specifically hybridizes to a target microRNA, because, as known by those of skill in the art, a mature microRNA does not contain a polyA tail. Therefore, there is no apparent reason why one of skill in the art would modify the teachings of Lau et al. to include a region to hybridize to a polyA tail when the target microRNA is known to lack a polyA tail. In fact, Spivack actually teaches directly away from the claimed invention with the teaching that the use of target specific primers are undesirable due to the requirement of very specific annealing conditions (page 2), the fact

that new reverse transcription primers would be required for each transcript target to be analyzed, and the inefficient use of RNA in target-specific analysis.

As noted in the present application, prior to the present invention, the amplification of small RNA molecules was difficult to those skilled in the art. As stated in the instant specification,

> Short RNA molecules are difficult to quantitate. For example, with respect to the use of PCR to amplify and measure the small RNA molecules, most PCR primers are longer than the small RNA molecules, and so it is difficult to design a primer that has significant overlap with a small RNA molecule, and that selectively hybridizes to the small RNA molecule at the temperatures used for primer extension and PCR amplification reactions.

See page 2, lines 20-25, of the specification published as International Publication No. WO 2006/081284.

Moreover, it is further noted that Spivack does not teach or suggest amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward primer and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions, as recited in Claim 21, as amended.

Therefore, it is demonstrated that a *prima facie* case of obviousness has not been established because (1) Brownie, Lau and Spivack, taken together or separately, fail to teach every limitation of the claimed invention; (2) there is no motivation or expectation of success to combine the references to arrive at the claimed invention because Spivack teaches directly away from the combination; and (3) the hypothetical combination proposed by the Examiner does not

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

result in the claimed invention. Accordingly, removal of this ground of rejection is respectfully requested.

The Rejection of Claims 9 and 11 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Brownie et al., *Nucleic Acids Research 25*(16):3235–3251 (1997) in View of Lau et al., *Science 294*:858–862 (2001), as Evidenced by Lau Supplemental Information, as Applied Above, in Further View of Crollius et al., *Nature Genetics 25*(2):235–238 (2000) and Buck et al., *Biotechniques 27*:528-536 (1999)

Claims 9 and 11, which depend from Claim 1, stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Brownie in view of Lau, in further view of Crollius et al., *Nature Genetics 25*(2):235-238 (2000) ("Crollius") and Buck et al., *Biotechniques 27*:528-536 (1999) ("Buck"). Applicant respectfully traverses this ground of rejection for at least the following reasons.

As described above with respect to Claim 1, as amended, Brownie and Lau, either separately or together, fail to teach or suggest every element of the claimed invention. Further, no motivation exists to combine these references and there is no reasonable expectation of success to achieve the claimed invention with respect to such as hypothetical combination. The teachings of Crollius et al. and Buck et al. fail to cure the deficiencies of Brownie and Lau in this regard.

The Examiner characterizes Crollius as teaching an oligonucleotide comprising the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:13. It is noted that there is no teaching or remote suggestion in Crollius with regard to a method using an extension primer that specifically hybridizes to a target microRNA for amplifying the target microRNA. Rather, Crollius et al. is directed to the use of pufferfish Tetraodon nigroviridis to detect conserved sequences in the

human genome with low background in order to estimate the number of genes in the human genome (abstract). The Examiner cites Buck et al. as disclosing evidence of the equivalence of primers. It is noted that there is no teaching or remote suggestion in Crollius et al. or Buck et al. with regard to the use of an extension primer that specifically hybridizes to a target microRNA. It is further noted that Crollius and Buck do not teach or suggest amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward primer and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions.

Therefore, it is demonstrated that a *prima facie* case of obviousness has not been established because (1) Brownie, Lau, Crollius, and Buck et al., taken together or separately, fail to teach every limitation of Claim 1, from which Claims 9 and 11 depend; (2) there is no motivation or expectation of success to combine the references to arrive at the claimed invention; and (3) the hypothetical combination proposed by the Examiner does not result in the claimed invention. Thus, as with Claim 1, these dependent claims are also not obvious over the cited art. Applicant respectfully requests withdrawal of this ground of rejection.

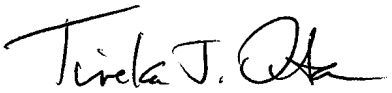
LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

CONCLUSION

In view of the foregoing amendments and remarks, applicant submits that all pending claims are in condition for allowance. Reconsideration and favorable action are requested. If any issues remain that may be expeditiously addressed in a telephone interview, the Examiner is encouraged to telephone applicant's attorney at 206.695.1655.

Respectfully submitted,

CHRISTENSEN O'CONNOR
JOHNSON KINDNESS[PLLC]



Tineka J. Quinton
Registration No. 53,496
Direct Dial No. 206.695.1655

TJQ:jlg

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS[PLLC]
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100